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SCREENING OF ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AND ITS CHEMICAL COMPOSITION AGAINST *MALASSEZIA FURFUR*

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ABSTRACT

This present study evaluates the potential antifungal activity of selected essential oils viz.; Tagetes minuta, Ocimum africanum, Eucalyptus citriodora and Mentha arvensis against Malassezia furfur. The studies were carried out using agar well-diffusion method at 50 µl. The minimum inhibitory concentration (MIC) were calculated using microdilution method and the volatile oil were analyzed by using GC-MS. Result confirmed the potential efficacy of essential oil of T. minuta, followed by E. Citriodora showing the maximum zone of inhibition (mm) 31.07 ± 2.01 and 24.75 ± 0.05, respectively, against M. furfur. The lowest MIC values of T. Minuta < 5 µl/ml was found to be effective against M. furfur. Essential oil of O. africanum was found less effective as it was unable to inhibit the growth of tested fungus. Results depict that the essential oil T. minuta may be used as an alternative to the other chemically made hair products.

KEYWORDS: Essential Oil, Malassezia Furfur, Lipase; Hydro-Distillation, GC-MS & MIC

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Original Article

1. INTRODUCTION

Malassezia furfur is a fungal species naturally found on the skin surfaces of humans and the most common secondary manifestation of seborrhoea on the scalp. Its alipophilic yeast requires fat to grow, characterized by a flaky and pruritic scalp, which affects half the world's population post-puberty [Turner GA, *et al*., 2012]. Several other skin-related problems caused by *Malassezia* species like pityriasis vesicular folliculitis, atopic dermatitis under climate and environmental conditions [Dawson T. L., 2006]. Dandruff appears when the natural renewal of cells is disturbed due to rapid growth of fungus and it causes itching too. The overgrowth of fungus may be related to increased oil production, hormonal changes, stress and neurologic disorders, [Ashbee H. R. and Evans E. G., 2002]. The available treatment of dandruff is antifungal cream and anti-inflammatory agents. Antifungal ketoconazole, zinc pyrithione and selenium disulphide have been found to be effective.

Essential oils are known for their potential source of antimicrobials of natural origin. It has been used in folk medicine throughout history. Numerous studies have been done on the antimicrobial activities of plants against many microbes, including food-borne pathogens [Ranganathan, S. and Mukhopadhyay, 2010]. Plant essential oil (volatile) is a hydrophobic liquid containing volatile aroma compounds. There are some major compounds, terpenoids and phenylpropanoids, which provide particular aroma and biological properties to EOs. In recent decades, with the popularity and scientific interest in essential oils of medicinal plant, aromatherapy has gained a great popularity, which is an alternative medicine that uses essential oils and other aromatic compounds. All over the world, traditional system prescribed essential oil as a solution for a variety of health problems. There are various pharmaceutical and biological activities like antibacterial, antifungal, anticancer, anti-mutagenic, antidiabetic,

antiviral, anti-inflammatory and antiprotozoal properties assigned to them. The aim of this study was to test various essential oils, viz., *Tagetes minuta*, *Ocimum africanum, Eucalyptus citriodora* and *Mentha arvensis* for antifungal activity against *M. furfur*.

2. MATERIALS AND METHODS

2.1 Essential Oil Extraction

For this study, fresh leaves of *Eucalyptus citriodora*, *Tagetes minuta*, *Ocimum africanum* and *Mentha arvensis* were collected from CSIR–CIMAP, Pantnagar. Leaves were washed and subjected to hydrodistillation for three hours to obtain essential oil. Distilled essential oils were collected separately in a tube and were dried using anhydrous sodium sulfate and stored at 4°C until tested.

2.2 Gas Chromatography

Nucon gas chromatograph model 5765 is equipped for the analysis of selected essential oil samples with a flame ionization detector (FID) and two stationary phases of different polarity, viz., BP-20 (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness) and DB-5 (30 m length × 0.32 mm internal diameter × 0.25 μm film coating) fused silica capillary columns. Hydrogen is used as carrier gas at 1.0 mL/min. From 70° - 230°C at 4°C/min temperature program is used with an initial and final hold time of 2 min (for BP-20) and from 70° - 250°C at 3°C/min (for DB-5). The temperature of injector and detector was 210°C and 230°C, respectively. Split ratio was 1:30.

2.3 Preparation of Test Organisms

Pure culture of *Malassezia furfur* (MTCC: 1374) was obtained from the Institute of Microbial Technology (CSIR), Chandigarh. The culture was maintained in SDA.

2.4 Antifungal Assay of Essential Oil

Agar well diffusion method was used to determine the zone of inhibition. The test organism *M. furfur* was inoculated in Sabouraud dextrose broth (SD Broth) and then incubated at 28^{o}C for 24 hours. Loop full of cultured organisms was diluted 10 times and 100 μl of diluted culture was spread over the surface of SDA plate with the help of sterile spreader. Wells were punched in agar surface by using sterilized cork borer (6 mm). To prevent the unwanted spreading of the extracts from the base of the wells, a drop of molten agar media was used to seal the base. 50 μl of undiluted essential oil of each plant was dispensed into the wells with the help of sterilized micropipette. The plates were kept outside for 30 minutes at room temperature and then incubated at 28^{o}C for 24–48 hours. Ketoconazole was used as a positive control. After 24 hours of active growth, the zones of inhibition were measured with the aid of a meter rule considering the diameter of the cork borer.

2.5 Minimum Inhibitory Concentration Test

The broth dilution technique was used to analyse MIC value of selected essential oil. The MIC was calculated only for those plant extracts that showed antimicrobial activity in the well diffusion assay. Various concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μl) of essential oils from the stock solution were prepared (1 ml of essential oil in 1 ml of DMSO) to perform the MIC test. 24-hour-old incubated suspension of *Malassezia furfur* in Sabouraud dextrose broth was taken in the test tube. Different concentrations were added to all the test tubes and were incubated at 28°C for 24 hours. After 24 hours of incubation, fungal growth inhibition was checked using a sterile cotton swab, which was dipped inside the suspension

broth and was then inoculated on SD agar. Growth of *Malassezia furfur* on solid media indicated that a particular concentration of extract was unable to inhibit the growth of fungus. The MIC was defined as the lowest concentration of an antimicrobial that inhibited the visible growth of a microorganism after overnight incubation. DMSO and Ketoconazole were used as negative and positive control, respectively.

3. RESULTS

3.1 Essential Oil Quality Analysis

Chemical analysis results of the tested essential oils are presented in Table 1. Essential oil of *E. citriodora* (lemon-scented gum), 69 compounds were identified representing the total oil compositions (93–98%). The enriched component found was citronellal (86.0%), followed by some other constituent *α*-pinene (22.0%), citronellol (11.9%) and *β*-pinene (4.6%). Altogether 28 constituents were identified in essential oil of *O. africanum* Lour which form 92–99% of the total oil compositions. Citral (55.0–75.5%), (*E*)-γ-bisabolene (2.3–9.2%), geraniol (1.5–6.5%) and linalool (1.1–6.0%) was identified as the dominant constituents. From representing 95.75% of the total oil compositions, *T. minuta* contain rich amount of dihydrotagetone (35.58%) followed by (Z)-tagetone (31.87%), (Z) – β- Ocimene (14.54%), Limonene, (E) – Ocimenone.

Table 1: Composition of Essential Oils of *Tagetes Minuta*, *Eucalyptus Citriodora*, *Ocimum Africanum* and *Mentha Arvensis*

Tagetes minuta		*Eucalyptus citriodora*		*Ocimum Africanum*		*Mentha Arvensis*	
Compound	**%**	**Compound**	**%**	**Compound**	**%**	**Compound**	**%**
Dihydrotagetone	35.58	*α*-pinene	22.0	Citral	55.0–75.5	Menthol	73.87
(Z) – Tagetone	31.87	citronellol	11.9	(*E*)-γ-bisabolene	2.6–9.5	p-Menthone	8.04
(Z) –β-Ocimene	14.54	*β*-pinene	4.6	Linalool	1.1–6.0	1-a-Terpineo	1.56
Limonene	4.82	β-caryophyllene	0.7–3.2	geraniol	1.5–6.5	3-Octanol	1.28
(E)-Ocimenone	2.48	α-humulene	0.4–2.7			Pulegone	2.43
		6-methyl-5-hepten-2-one	≤0.03–2.1				

3.2 Determination of Anti-Fungal Activity

All tested essential oil has shown effective results against *M. furfur* except the essential oil of *O. africanum*, which failed to inhibit the growth of fungus (Table 2). Yellow colour essential oil of *T. minuta* exhibited the highest activity with inhibition zone diameter of 31.07 ± 2.01 mm against *M. furfur*. Significant antifungal activity was observed in *E. citriodora* essential oil showing 24.75 ± 0.05 mm zone of inhibition. Essential oil of *M. arvensis* showed moderate activity against M. furfur. On the other hand, *O. africanum was* found sensitive with a little inhibition zone diameter 10.01 ± 0.01 mm when the results were compared to control ketoconazole, which showed 23.56 ± 1.20 mm was less than the essential oils *T. minuta* and *E. citriodora*. No inhibition zone was observed in negative control DMSO.

Table 2: Determination of Antifungal Activity and MICs Value Against *M. Furfur*

Essential Oils	**Inhibition Activity Against *M. furfur***	
	Antifungal Activity (mm)	**MIC (*μ*l/ml)**
Tagetes minuta	31.07 ± 2.01	<5
Eucalyptus citriodora	24.75 ± 0.05	<15
Ocimum africanum	10.01± 0.01	-
Mentha arvensis	22.44± 1.26	>25
ketoconazole	23.56 ± 1.20	>20
DMSO	-	-

3.3 Minimum Inhibitory Concentration (MIC)

The MIC result of all the essential oils was tested against *M. furfur,* presented in Table 2. MIC assay was not performed due to one essential oil of *O. africanum* due to its less or no activity against the fungus. *T. minuta* showed the maximum activity at lowest concentration of < 5 μl/ml. *M. arvensis* oil needs higher concentration (> 25 μl/ml) to inhibit the growth of *M. furfur*. Lowest concentration of *E. citriodora* essential oil at which the growth of *M. furfur* was inhibited completely < 15 μl/ml.

4. DISCUSSIONS

Fungal invasion causes infections, substantial morbidity and mortality. There are so many health issues, resulting in a high cost for healthcare. There are few reports concerning the susceptibility of *Malassezia* to natural antifungal or anti-*Malassezia* agents [Gupta, A. K. *et al*., 2004]. Consequently, plant-derived anti-fungal agents are of increasing interest for the development of new, more effective and specific anti-*Malassezia* agents. In modern days, a number of drugs are developed from plants, which are found active against many diseases [Ayyanar, M. *et al*., 2009]. In this study, essential oils of different plants were tested for antifungal activity against *M. furfur*. According to Table 2, *T. minuta* essential oil was resistant to the fungus, which might be possibly due to the presence of four monoterpene constituents — limonene, β-Ocimene, dihydrotagetone and tagetone. Dihydrotagetone has been cited as one of the most abundant constituents of *T. minuta* oils from plants sampled from a wide range of countries such as Kenya [Garcia, M. V. *et al*., 2012]. Many previous studies and experiments were reported on the antifungal activity of plant essential oils against dandruff causing fungi *Malassezia furfur* [Lee *et al*., 2010 and Arora Pooja *et al*., 2013]. In one of the studies, *Eucalyptus* ***globulus*** (blue gum), *Phyllanthusemblica* (Amla) and ***Wrightia tinctoria*** (pala) leaf extracts and oil were tested, which showed antifungal property, as they progressively inhibited the growth of *M. furfur* on Sabouraud's dextrose agar medium [Vijayakumar, R. *et al*., 2006]. In general, for treatment of dermatological disorders including dandruff/seborrheic dermatitis and pityriasis versicolor, azole drugs such as fluconazole and ketoconazole are used, but with increasing usage of antifungal agents, those that have led to undesired effects include severe toxic hepatitis, acquired cutaneous adherence [Polsen, J. A., *et al*., 1995]. When comparing the previous result with the present, positive control ketoconazole showed less effectiveness than the selected essential oils of *T. minuta* and *E. citriodora*.

5. CONCLUSIONS

In this study, the results confirm that all essential oils tested clearly exhibited antifungal activity except *O. africanum* essential oil. From the result, *T. minuta* essential oil can improve stratum corneum while providing the strong anti-dandruff activity. Further studies are necessary, which provide a better understanding in the action of essential oil used and give us an effective alternative to synthetic products and therapeutic treatments.

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